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BY ROBERT H. BOWEN.

FROM THE DEPARTMENT OF ZOÖLOGY, COLUMBIA UNIVERSITY.

WITH TWO PLATES.

(Continued from page 3 of cover.)

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Introduction.

In my preliminary "Study" on the formation of the hemipteran sperm (Bowen '20), a summary was given of the occurrence of *polymegalous* spermatocytes and sperms in the Family *Pentatomidae*, together with a brief statement of the processes by which the polymegalous sperms are differentiated. It is the purpose of this paper to amplify my original account, thereby concluding the detailed description of hemipteran spermiogenesis which was begun in the second and third articles of this series (Bowen '22*a* and *b*).

The material, as indicated by the title, was drawn entirely from Hemiptera belonging to the Family *Pentatomidae*. Illustrative material has been drawn from a great variety of genera; but, as in my second paper (Bowen '22*a*), the details of sperm formation were worked out only in *Murgantia histrionica* Hahn, the testes of which were fixed for one hour in strong Flemming and subsequently stained with Fe-hamatoxylin and light green. For studying mature sperms in their entirety, smears were made substantially in accordance with the method of Faust ('13), osmic fumes, Gilson or Bouin being used for fixation, followed by Fe-hematoxylin, sometimes with a counterstain. In preparing these smears only the vas deferens was used,—a precaution which insures the exclusion of immature sperms. The preparations examined for the occurrence of *polymegaly* were fixed for the most part in Flemming and stained in Fe-hematoxylin. Wherever possible, the comparisons of various cell elements were carried out on such preparations, but for certain purposes it was necessary to employ special methods which are indicated in the explanatory text accompanying the figures.[1]

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[1] For a more complete treatment of technical methods see Bowen '22*a*.

The carrying out of this study on a comparative basis was made possible by the comprehensive collection of preparations of hemipteran germ cells which Professor E. B. Wilson had accumulated during his years of chromosome study. This entire collection he very kindly placed at my disposal, and for its use I am greatly indebted to him. I am further indebted to Professor Wilson for many valuable criticisms, appreciation for which I wish to express in this place. I am again indebted to Mr. H. G. Barber for the identification of much material of my own collecting which was employed for checking and other purposes.

Observations.

I. The Phenomena of Polymegaly.

Hemiptera of the Family *Pentatomidae* possess two compact testes, presenting typically a rather oblong contour when seen in the proper longitudinal section (Text-figs. 1*A* and *B*). Each testis is enclosed in a connective tissue sheath which is continued into the body of the gland as septa or partitions dividing it into a number of compartments, or, as I shall call them, *lobes*, arranged parallel to the long axis of the testis. These lobes vary in number from three to seven (in the forms examined by me,— see Table I), but for any particular species the number is constant. The lobes are typically arranged side by side in a single series (Text-figs. 1*A* and *B*), each lobe passing from one side of the testis to the other (Text-fig. 1*C*); but in some cases (*Perillus* (= *Mineus*) *bioculatus* for example) a tendency for the lobes of the testis to be bunched together is evident, and in *Stiretrus anchorago*, the serial arrangement is completely lost, all the lobes being visible at one time only in cross-sections (Text-fig. 1*D*). In a few cases of this kind, some of the lobes appear to have arisen by the subdivision of an originally single, typical lobe. In any case, the lobes all open at one end into a common collecting chamber, from which the efferent duct (*d* in the figures) of the testis is given off. In the typical case, this duct arises laterally (Text-figs. 1*A* and *B*), but in *Apateticus crocatus*, for example, it is more nearly median, and in *Elasmostethus cruciatus* it is quite central, the testis spreading out fanwise like that of a coreid. *Euschistus* and *Murgantia* are good examples of the typical arrangement, and Text-figures 1*A* and *B* give an idea of the general topography as seen in longitudinal sections of the testis of these bugs. It will be noted that the lobes differ in width, and these differences are in general definite and specific ones, which

may be accompanied by still other peculiarities of constant occurrence (see Montgomery '10). Within each lobe the cells are grouped in *cysts* which are arranged as a rule in a double [2] series (as seen in longitudinal sections like that of Text-figures 1*A* and *B*) throughout the length of the lobe, the various stages following one another in an accurate series, beginning at the blind end of each lobe with spermatogonia. At any given level the cysts of each lobe are in about the same stage (the large spermatocyte generations introduce some discrepancies) so that in a given testis it is usually possible to find in one lobe or another every step in the spermatogenesis. When the cysts of

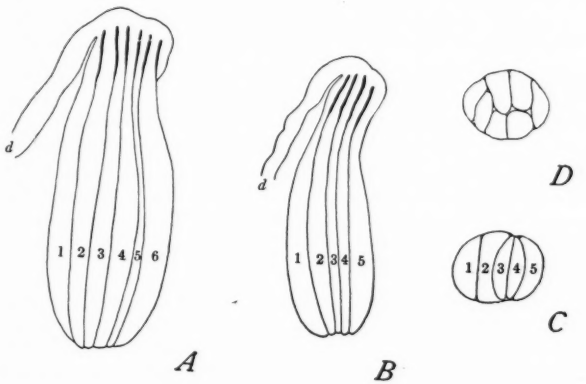

FIGURE 1. Structure of the pentatomid testis (x18). Lobes numbered as described in the text; *d*, efferent duct. (Flemming-hematoxylin). *A*, longitudinal section of the testis of *Euschistus servus; B*, the same — *Murgantia histrionica; C*, cross-section of the testis of *Murgantia histrionica; D*, the same — *Stiretrus anchorago.*

completed sperms reach the open ends of the lobes, the sperms are emptied into the collecting chamber, whence they pass into the efferent duct.

The occurrence of morphological differences between the lobes or their contents makes some system of nomenclature desirable, whereby reference may readily be made to any particular compartment in a testis. For this reason I have adopted the plan [3] of numbering the

[2] In large-celled lobes the cysts are often arranged in a *single* series.

[3] This method of designation was used by Montgomery ('98) who appears to have numbered the lobes quite arbitrarily, his numerical order being the

lobes consecutively, reckoning the first on that side toward which the efferent duct opens as lobe 1, lobe 2 the next, and so on, as indicated in Text-figures 1*A*, *B* and *C*. In certain special cases, *e.g.*, *Stiretrus* (Text-fig. 1*D*) this plan can not be rigorously applied, and in such cases, a more or less arbitrary numbering must, of course, be adopted.[4]

In 1898 Montgomery called attention for the first time to the fact that in *Euschistus* (probably *tristigmus* and another species (Montgomery '10), though both were referred originally to *Pentatoma* and *Tropicoris*) lobes 4 and 6 of the testis were characterized by the constant possession of spermatocytes unusually large as contrasted to those of lobes 1, 2, 3 and 5. In lobe 1, the spermatocytes averaged slightly smaller than in those of lobes 2, 3 and 5, but the difference was not considered significant. The structure of all the spermatozoa was stated to be the same, though differences in size were inferred. This peculiar polymorphism is not due to any of the known nuclear derangements, in fact the cells in all lobes are identical up to the synaptic period. As the spermatocytes enter the growth period, however, the nucleus and cytoplasm of those in lobes 4 and 6 increase in size very much more than the others, resulting in characteristically "large" generations. Nevertheless, in the reduction divisions all the chromosome plates are identical in composition and corresponding chromosomes are of exactly the same size regardless of the disproportion in the sizes of the cells themselves. Montgomery was disposed to attribute the greater size of the cells in lobes 4 and 6 to nutritional differences traceable to the blood supplies. He also noted some specific differences in the cytoplasmic structures to which reference will be made elsewhere.

In 1910, Montgomery published a paper dealing in part with this unusual "dimegaly,"—as he now termed it,—including observations on the mature sperm and further notes of a general nature. In this paper he repeated his former statements as to the size relationships, but now reported that in lobe 5 the spermatocytes were constantly smaller[5] than in lobes 1, 2 and 3. No reference was made to his

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reverse of mine. Some systematic method of orientation is, however, essential in examining many different species, and as the sperm duct is a constant landmark I have found it convenient to number the lobes in relation to it. I shall express Montgomery's findings in my own system of numbering, a fact to be remembered in comparing with his original reports.

[4] For other details of the structure of an hemipteran (*Euschistus*) testis, reference may be made to Montgomery's papers, especially that of 1898.

[5] I shall refer to the three sizes of cells as (1) large or unusually large, (2) normal or small, and (3) smallest or unusually small.

earlier statement that a smaller generation occurred in lobe 1, an inaccuracy due, perhaps, to a typographical error. His later description is certainly correct for *Euschistus variolarius* and *E. euschistoides* and probably for all the other species of the genus as well. Montgomery now considered the dimegaly as "due directly to differences of the nurse cells of the different follicles, *i.e.*, to degree of nutrition."

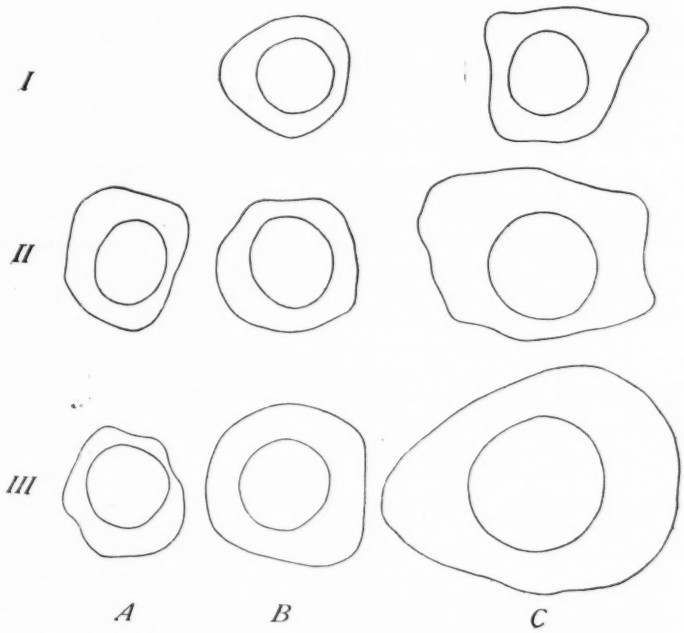

FIGURE 2. Outline drawings showing extent of nucleus and cytoplasm in primary spermatocytes of the late growth period (x1450). (Flemming-hematoxylin.) Row I, *Murgantia histrionica;* Row II, *Euschistus variolarius;* Row III, *Arvelius albopunctatus.* Columns *A*, *B*, *C*,— cells of the smallest, small or normal, and large generations respectively.

He also added definite proof of size differences in the heads of the sperms, showing from sections that each of the three spermatocyte generations gave rise to sperm the heads of which were likewise of three sizes. That these general size differences are constant and not

mere chance variations was proved by examination of 41 testes of *Euschistus sp.*, and 4 testes of *E. tristigmus*, in all of which the conditions were identical. Certain other pentatomids (*Brochymena, Perillus, Nezara, Cosmopepla, Mormidea, Trichopepla*) were also examined, but in no case did Montgomery find "such constant size difference," or at least it was "much less appreciable." In his final paper on *Euschistus*, Montgomery ('11) intimated that he had made further observations on the phenomenon of "dimegaly" in *Euschistus*, but these were never published and the subject has not since been reëxamined.

My attention was attracted to this problem by the discovery that in *Murgantia* size differences also occur, very much less conspicuous than in *Euschistus*, giving rise to cell generations of two sizes,—the spermatocytes in lobes 1, 2 and 5 (Text-fig. 1*B*) being small or "normal," in lobes 3 and 4, somewhat larger. Text-figures 2–I give an idea of these size relations in the primary spermatocytes, which may be compared with the corresponding cells of *Euschistus* shown in Text-figures 2–II. It was found subsequently that the expected two sizes of sperms (Text-fig. 3) were formed by processes differing somewhat superficially, but as constant as the dimegalous condition itself. These facts, taken together, aroused the suspicion that this whole phenomenon might not be so unique as Montgomery had thought, and I accordingly undertook to make as complete a survey as possible of the Family *Pentatomidae* with respect to this particular point. Such an examination was made possible by the fact that Professor Wilson's preparations,—referred to above,—included a large number of pentatomids of which the majority had received dependable identification. My results are tabulated in

FIGURE 3. Mature sperms from the vas deferens of *Murgantia histrionica* (x965). (Smear preparation; osmic fumes-hematoxylin.) *A*, from the small or normal generations; *B*, from the large generations.

Table I, into which some errors have doubtless crept through insufficient material, accidents of sectioning, or doubtful synonymy of the generic titles. Where the material was obviously insufficient or unsatisfactory the fact is indicated by a dash, and doubtful points are marked "?." It should be added that, like Montgomery, I have as a rule recorded size differences only when they were reasonably obvious. In other words, a case like *Murgantia* in which the size differences are slight, might be overlooked, especially if the material was not first class. Furthermore, I have made no special effort to detect lobes in which the spermatocytes are unusually small. This was because the differences in this respect are by no means striking,— in fact are often rather difficult to make out even in *Euschistus* if the material is poor,— and the value of the results seemed hardly commensurate with the labor involved.

The table gives in the first column the list of forms examined, the nomenclature followed being that of Van Duzee ('17); in the second column is given the number of lobes in the testis of a given species, while the third and fourth columns give the index numbers of the lobes in which unusually large or unusually small (where noted) cells occur, these numbers being assigned in accordance with the plan previously described (see Text-figs. 1*A* and *B*). Comparisons were generally made on spermatocytes in the late growth period, and exact observations on the mature sperm were usually impossible for obvious reasons.

Examination of Table I shows at once that far from being a phenomenon unique to a single genus, the spermatic polymegaly is widely distributed in the Family *Pentatomidae*, and is in fact rather more common than rare. Montgomery's original idea is therefore erroneous, and his failure to detect differences in some of the other genera which he examined for this point is rather puzzling. Thus in our common species of *Brochymena* the difference is very striking, though Montgomery reported a general equality for this genus. Possibly a clue is to be found in the case of *Nezara*, in which no dimegaly occurs in the northern species, *hilaris*, but a very noticeable one in the southern species, *viridula*. At the same time it is interesting to note that Van Duzee ('17) has now placed these two species in different genera, *Nezara* and *Acrosternum*. Doubtless the form which Montgomery examined was *Acrosternum* (= *Nezara*) *hilaris*, in which absence of dimegaly is also indicated by my observations. Further, in a number of known cases the identification of forms studied by Montgomery ('98 and '10) was not very critical, and the discrepancies may be due in part to such errors.

TABLE I.

Classification of forms examined (After Van Duzee '17)	Number of lobes in a single testis	Lobes in which cells are unusually large	Lobes in which cells are unusually small
Family PENTATOMIDAE			
Subfamily *PENTATOMINAE*			
Tribe *HALYINI*			
Brochymena 4-pustulata	7	4,6	
Tribe *PENTATOMINI*			
Peribalus limbolarius	6	0	
Trichopepla semivittata	7	0	
Rhytidolomia saucia	6	3,5	
" *senilis*	6	3,5	
Chlorochroa uhleri	6	3,5	
Carpocoris sp.	6	3?	
Solubea pugnax	4	0	
Euschistus servus	6	4,6	5
" *euschistoides*	6	4,6	5
" *inflatus*	6	4,6	5
" *tristigmus*	6	4,6	5?
" *variolarius*	6	4,6	5
" *ictericus*	6	4,6	5
Coenus delius	6	4,6	5
Aelia americana	7	–	
Cosmopepla bimaculata	5	?	
Thyanta custator	4	0	3?
" *calceata*	4	0	3?
" *casta*	6	3,5	
Loxa florida	7	4,6	5
Murgantia histrionica	5	3,4	0
" " (var. *nigricans*)	5	3,4	0
Nezara viridula	6	3,5	4
Acrosternum hilaris	6	0	
Banasa dimidiata	3	0	
" *calva*	3	0	
Piezodorus guildinii	5?	0	
Arvelius albopunctatus	6	3,5	4
Tribe *EDESSINI*			
Edessa bifida	5	2,4	
Subfamily *ACANTHOSOMINAE*			
Elasmostethus cruciatus	7	0	
Subfamily *ASOPINAE*			
Stiretrus anchorago	7	0	
Perillus bioculatus	7	0	
Euthyrhynchus floridanus	6	–	
Apateticus crocatus	7	0	
Podisus maculiventris	7	0	

The results obtained do not appear to be susceptible of any logical arrangement, for between species of the same genus, e.g., *Thyanta*, great differences may occur. On the other hand, in *Euschistus* the differences seem to be similar (though perhaps varying somewhat in amount) throughout the series of species examined. It is evident, therefore, that no obvious and constant correlation exists between the occurrence of polymegalous spermatocytes and generic relationships, in which respect these cellular differences parallel the similar lack of correlation between chromosome number and grouping, and external characters which has now been observed in a number of cases. Nevertheless, it is a striking fact that among insects these particular differences occur only in this single family, so far as I know, and whatever the cause of their origin may have been, it was doubtless the same for all the cases. Possibly we have to deal with a case of parallel mutation. In line with this apparently capricious occurrence of polymegaly is the absence of any conspicuous regularity in the particular lobes affected. On the other hand, there is a curious tendency for the lobes with large cells to occur in pairs, the components of which are separated by a third lobe which often contains the smallest generation of cells; and, further, these large-celled lobes tend to be located on the side of the testis opposite that from which the efferent duct takes it origin. With respect to their comparative morphology, I have noted that the large-celled lobes are frequently narrower than the others (see Text-figs. 1*B* and *C*), while the lobes with unusually small cells are occasionally of exceptional size. There is, therefore, no direct relation between the sizes of the lobes and the cells they contain.

One of the most interesting points brought out by this survey is that the degree of size difference may vary greatly in different genera, so that a whole series might be arranged running in graded order from the complete absence of this phenomenon, to very exceptional cases of size difference. Thus in *Banasa*, *Acrosternum*, and other genera there seems to be no visible polymegaly; in *Murgantia* (Text-figs. 2–I) the difference, though small, is usually readily distinguishable, but by no means striking; in *Euschistus* (Text-figs. 2–II) and *Brochymena* the difference is conspicuous; while, finally, in *Arvelius* (Text-figs. 2–III) it is truly extraordinary, the whole testis being dominated by the generations of large spermatocytes beside which the normal and smallest generations are completely dwarfed. This last mentioned genus deserves special comment for in *Arvelius* the phenomenon of polymegaly reaches its greatest development. In fact, the volume of the large primary spermatocytes is something like eight

times that of corresponding cells in the smallest generation. This enormous difference is of special value in determining the size relations of various cellular components and I have constantly taken advantage of this unusual opportunity for critical comparisons. I should further state that there is in *Arvelius* very good evidence of a fourth generation of cells occupying lobe 2, which are slightly larger than those in lobes 1 and 6. My insufficient material does not, however, allow a definite statement on this point. It thus seems not improbable that a pentatomid genus may be found in which each of the lobes would differ from all the others in respect to the relative size of its cells. In line with these comments which refer particularly to the large generations, it may be added that rarely the smallest generations of cells are distinguished by their unusual size relations. Thus in *Loxa florida* the smallest cells are strikingly smaller than those of the normal and large size.

(*a*) *The effects of polymegaly on various cellular constituents.*— It will be convenient here, before going on to an examination of the size differences in the sperms, to consider in detail the possible differences in the spermatocytes and their maturation divisions which might be dependent upon the size relationships of the cells in different lobes.

As Montgomery ('98) first showed, all the cells in all the lobes seem to be in every way identical and normal up to the period of synapsis which directly precedes the so-called growth period of the primary spermatocytes. The one marked difference is in the number of cells which are in any one of these early stages. In the large-celled lobes the cells are much less numerous than in the lobes with smaller cells due apparently to the lack of room, the available space being much curtailed by the larger size of the cells in later stages and by the added fact that the large-celled lobes are often of markedly smaller volume, as noted above. In the growth period the size differences quickly become apparent and the various generations of sizes become more and more clearly marked, every [6] cell being equally involved at any particular stage. The differential factor of growth seems to be one of *quantity* only, rather than *quality*. It will be interesting to inquire into the behavior of the various cell elements in large and small sperm cells.

[6] I have not met with a single case of failure to develop the polymegalous inequalities customary for a given genus, and only one case (a specimen, No. 94, of *Euschistus*) has been found in which all the cells were not affected to the normal degree. In this particular case (which was also abnormal in some other respects) scattered cysts occurred among the *large* generations (in both lobes 4 and 6) in which the cells were all *normal* in size. Cysts of this kind were found in primary spermatocyte, and spermatid stages. There was no clue to their method of origin.

Respecting the general proportion between cytoplasm and nucleus in the large- and smaller-celled generations, Montgomery was of the opinion that the increase in size was due primarily to added cytoplasm and in a lesser degree to the volume of the nucleus. My own observations, especially on *Arvelius*, do not bear out these conclusions entirely, and while the nucleo-plasmic ratio seems to be slightly reduced in the large spermatocytes, I do not think that the reduction is a particularly striking one. (Compare especially Text-figs. 2–III*A* and *C* and Figures 1 and 2.) More exact measurements of the nucleo-plasmic ratios would be of interest, and they could readily be obtained by computing the proper areas on many camera lucida drawings by means of a planimeter.

As to the contents of the nucleus, I agree with Montgomery that the karyolymph, linin and plasmosome are increased in volume in the large cells, but I can not corroborate his statement that, "the chromatin nucleolus" (X and Y chromosomes) "is ... larger in cells of the large than in those of the small generation." This statement seems to me doubtful. The plasmosome or true nucleolus is often stained quite differently in the large and small cells, the result apparently of differences in the rapidity of extraction (Figs. 1 and 2). With respect to the most important constituent of the nucleus, the chromatin, Montgomery pointed out that its amount was the same in all nuclei regardless of size. This is indicated in part by the difference in appearance of the large and small nuclei during the height of the growth period, the former being relatively poorer in chromatin as indicated by their 'clearness' (compare Figs. 1 and 2). As several workers have shown, the diplotene threads resulting from synapsis, become spread out in a "confused" way during the growth period, the separate chromosomes being thus lost from view for a time. In the large cells this confused state reaches a very advanced stage, while in the smallest nuclei it scarcely gets a start. Indeed, it is possible that in such nuclei (in *Arvelius*, for example) the threads could be followed through without a break to the prophases of the first maturation division. The unusual diffusion in the confused period in the large cells has brought to light a very interesting phenomenon in connection with the behavior of the sex chromosome nucleoli (thus far I have studied this in *Euschistus* only) during the earlier part of this stage. These bodies, which are usually stated to retain their definite, compact form (and were so described by Montgomery '11), become broken up into groups (of more or less definite individuality) of granular masses. These may be arranged in a chain formation, or simply massed in an in-

definite cluster. Subsequently they become reunited to form the characteristic chromatin nucleoli of the later growth period. This same phenomenon occurs also in the normal cells, but the tendency toward diffusion is here less marked and the fragmentation of the chromosome nucleoli correspondingly less conspicuous. Indeed, it was not noted by Montgomery ('11), who merely states that the contour of the nucleoli at this time may be "irregular." In this connection Wilson's ('12) figures (figs. 100 *c, d, f*) of the granular structure of the sex chromosomes (nucleoli) of *Lygaeus* in the growth period are very suggestive.

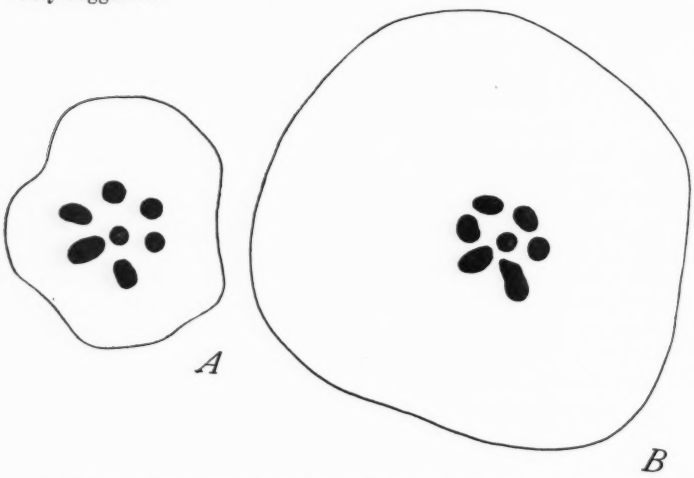

FIGURE 4. Metaphase chromosome plates of the second maturation division in *Arvelius albopunctatus* (x3050). The limits of the cytoplasm are indicated by a simple line. (Flemming-hematoxylin.) *A*, from the smallest generation; *B*, from the large generations.

Except for these minor differences in the extent of diffusion, the chromatic history in all generations of primary spermatocytes is identical, and when the maturation division figures are formed, the chromosome plates are likewise identical. *Arvelius* furnishes excellent material for the study of these stages. In this bug the behavior of the chromosomes is similar to that of *Euschistus* (see Montgomery '11), the first maturation spindle having six autosomes with separate X and Y chromosomes, while the second has a ring of six autosomes enclosing an X–Y dyad. The latter is shown in Text-figure 4. Not only are the chromosomes the same in number in all

cells, but specific ones which can be identified by reason of various individual peculiarities can be readily recognized in both large and small generations. Similarly, the metaphase plates are roughly alike in area, though the chromosomes may be sometimes slightly more spread out than at others. Such is the case in Text-figure 4*A;* but other plates of the same kind are often more compact. The point of importance is that the plates are of approximately equal extent (absolute, not relative), so that regardless of the amount of cytoplasm and the length of the spindle the chromosomes in the large spermatocytes form a metaphase plate essentially like the one they would have formed in a cell of very much smaller volume (compare Text-figs. 4*A* and *B*). On the other hand, the spindle lengths (measured from centriole to centriole at metaphase) are approximately proportional to the size of the cells. I have made a long series of measurements (with a filar micrometer) of the spindles in *Arvelius*, with the results shown in Table II. Examples from the large cells were scanty, but sufficient to establish the general proposition beyond question. Finally, and perhaps of most interest, *the sizes of corresponding chromosomes seem to be the same in all cells regardless of their size.* It was upon this point that Montgomery placed special emphasis. I have tried to check his results critically in *Arvelius*, which is a much more favorable form than *Euschistus* for this purpose. My results failed of a rigorous demonstration of chromosome equality, in part because of inadequate material but chiefly because of the lack of any sufficiently precise method of measurement. Nevertheless, disregarding the slight and accidental differences which are bound to appear in such direct comparisons as those of Text-figures 4*A* and *B*, it appears that the corresponding chromosomes are of sizes which, if not exactly identical, certainly correspond within very small limits of difference.

TABLE II.

	Smallest	Normal	Large
1st Mat. Div.	0.9	1.0	1.8
2nd Mat. Div.	0.7	1.0	1.7

Ratio of spindle lengths. The average length of the spindle in a cell from the "normal" generation is taken as unity (1.0).

The enormous increase in the amount of cytoplasm in dimegaly has already been noted and it remains now to examine the behavior of its various formed elements. It might naturally be supposed that in a

process affecting the cytoplasm so vitally, its several components would develop morphological differences (other than those of mere size or amount) of one sort or another. Such, however, is not the case, and with the exception of a few minor differences upon some of which I have already touched (Bowen '20), the general progress of events in the cytoplasm is the same in all cells regardless of size. With respect to staining reactions and the response to impregnation methods involving silver nitrate there are, however, some rather definite differences between the large and small cells, but these are perhaps referable to a physical rather than a physiological difference.

The *centrioles* were stated by Montgomery ('98) to vary in size directly with the amount of cytoplasm, but his figures were not entirely convincing. I have reëxamined this point in *Arvelius*, in which the facts are demonstrated with unusual clearness. The centrioles in this form are rod-like in shape, often arranged in the primary spermatocytes in a "V" formation recalling the condition in Lepidoptera. The centrioles in the large generation are strikingly larger than in the smaller generations. (Compare Figs. 1 and 2, in both of which the size of the centrioles has been slightly exaggerated).

The *mitochondria* were found by Montgomery (he at first called the mitochondrial material an idiozome) to vary in amount with the cytoplasm. This is easily checked by comparing the large and small spermatids during the stage when the mitochondria are aggregated into the compact, rather accurately spherical nebenkern. (Compare Figs. 3 and 4).

The *Golgi apparatus* was not considered by Montgomery. In its scattered condition in the spermatocytes (see Bowen '20) comparisons are not very satisfactory, but after the aggregation of the Golgi elements to form the acroblast in the spermatid, the large and small generations can be readily contrasted (Figs. 7 and 8). As with the mitochondria and the centrioles, the large cells have much the larger amount of Golgi material. So too, the acrosome formed in connection with the acroblast is much larger in the large cells, sometimes very strikingly so (Figs. 9 and 10), and the cast-off acroblast (Golgi remnant) varies of course in a similar way (Figs. 9 and 10).

The *chromatoid body* is another rather constant feature of the pentatomid germ cell which was not considered by Montgomery. This structure does not lend itself easily to comparison because of its small size, and often subdivided condition. However, in *Rhytidolomia senilis*, as discovered by Wilson ('13), the chromatoid body is very large and distinct, and makes a fine object for comparison (Figs. 5 and

6). Further, in this species, the chromatoid body is shaped like a thick disc, the periphery of which is roughly circular, a peculiar shape which makes possible the determination of the long diameter without difficulty, and I have made a series of measurements of that dimension in both large and small cells in spermatocyte, division, and spermatid stages. These show that the diameter of the chromatoid body in the small cells differs from that in the large ones in the ratio (approximately) of 1.0 to 1.2. The thickness of the disc is also increased in the large cells. My observations show, therefore, that the chromatoid body varies in size with the cell as a whole, but seem to indicate at the same time that the increase in size is not exactly proportional to that of the cytoplasm in this particular species.

It appears, therefore, that all the cytoplasmic components are increased in amount (or size) in the large cells, and that the chromatin content is accordingly the only known constituent in the entire cell which is not affected in polymegaly. This is a point of extraordinary interest, and, taken in connection with the fact that this condition occurs in the germ cells, furnishes an interesting side-light to the great mass of evidence already accumulated that the chromatin plays the leading rôle in hereditary transmission. Furthermore, the increase in the volume of the formed elements of the cytoplasm in the absence of a corresponding increase in the volume of chromatin in the nucleus suggests a number of interesting possibilities as to the nature of the relation existing between the chromatin and the cytoplasmic constituents. Apparently the volume of the cytoplasmic constituents cannot be considered as a simple function of the volume of the chromatin in a given cell.

(*b*) *The effects of polymegaly on mature sperms.* — As stated above, Montgomery found in the mature (?) sperms of *Euschistus* three sizes of heads varying in length according to the size of the spermatocyte generation from which they originated. His observations were on sections only, so that determination of the total dimensions of the sperm were impossible. I have worked out the "dimegalous sperm" of *Murgantia* in much more complete form, and some account of their differentiation will be given later; here I wish merely to point out that two sizes of sperms occur corresponding to the two generations of spermatocytes. In sections, the actual origin of the two classes of sperms can be positively traced to the two classes of spermatocytes, and by means of smears, the two kinds of sperms can be readily isolated entire. Text-figure 3 gives a general idea of the contrast in structure. The two classes are unexpectedly of about

equal total length, but the large sperm have a much heavier tail, near the tip of which is a characteristic swelling. The head, on the other hand, is exceedingly long but correspondingly decreased in diameter, so that it is scarcely more than a thread. Smears of *Euschistus* were also made, in which the three expected kinds of sperm were very easy to distinguish, the large ones proving of extraordinary size. Text-figure 5 gives an idea of the comparative sizes of the three sperm classes. In these figures the diameter of the sperm head has been much enlarged in each case in order to make the heads clearly evident at such low magnification. Actually, the large sperm head is exceedingly delicate and thread-like, and not at all like the figures given by Montgomery ('10; figs. 24–26). According to Montgomery the volume of the large and small sperm heads differs markedly, and this difference was supposed to depend on the fact that the chromatin exists in the sperm head as a peripheral layer enclosing a quantity of karyolymph, the latter determining the size of the head. My observations do not corroborate these statements in any particular. As will be pointed out in a later section, and as I have shown previously (Bowen '22*a*) for the small sperm of *Murgantia*, the head is probably a solid rod of chromatin, and the appearance described by Montgomery (if in *mature* sperms) is due to faulty technique. As a matter of fact, in sections such as he used the heads are often made to appear thicker than they actually are. As indicated by smears, the volumes of the mature sperm heads are probably very similar, allow-

FIGURE 5. Mature sperms from the vas deferens of *Euschistus euschistoides* (x255). (Smear preparation; osmic fumes-hematoxylin.) *A*, from the smallest generation; *B*, from the small or normal generations; *C*, from the large generations (the tail has been bent in the drawing in order to save space.)

ance being necessary, however, for the expanded basal end of the head, which is probably to be viewed as a "centrosomal middle-piece" (see Bowen '22*a*).

Unlike *Murgantia*, in *Euschistus* the length of the sperms as a whole also differs markedly, the actual length of the large ones being about 1 mm. These large sperms are not easy to find entire, as they are usually broken or tangled up with others in making the smear, but one can always be certain of an entire sperm by the presence of the thread-like head and the characteristic swelling near the tip of the tail. Smear material from *Arvelius* was unfortunately not available, so that I have been unable to determine the length of the large sperms. If they vary in size as in *Euschistus*, (and sections at hand seem to indicate that they may), the large sperms would be one of the largest animal sperms recorded.[7]

These results have an unexpected bearing on the attempts made some years ago by Zeleny and his pupils to establish quantitative differences in the length of the sperm head referable to differences in the volume of the sex (X and Y) chromosomes. As a matter of fact, their plotted measurements of sperm heads did give a bimodal curve in many cases, and this was held to be proof of the original contention. Without undertaking a critique of this whole conception, it may be pointed out that differences arising from polymegaly, such as I have here described, were not taken into account; yet they would obviously introduce a fatal source of error. Nor is it sufficient to say that because visible differences in the spermatocytes are not readily to be detected, therefore, none occur in the resulting sperms; for in *Murgantia*, to cite an instance, the spermatocytes are not always easily separable on the basis of size, and yet the spermatic differences are very considerable. It is easily conceivable that a pentatomid might be found in which polymegaly of the sperm would be distinguishable, and yet size differences in the spermatocytes might be to all intents and purposes lacking. Some of the forms classed in Table I as failing to show polymegaly may, indeed, belong to just such a type. Polymegalous differences of a minor type may possibly be much more common than is suspected. As a matter of fact Zeleny and Senay ('15) did measure the sperm of *Euschistus variolarius*, obtaining a strikingly bimodal curve, in which, however, the ratio of difference was very much more than it should have been according to the

[7] The largest animal sperm on record seems to be that of *Notonecta*, which also happens to be an hemipteran. Pantel and de Sinéty ('06, page 89) give its length as 12 mms. and over.

theoretical calculations. The discrepancy was never satisfactorily explained, so far as I know. The facts here given concerning the sperm heads of *Euschistus* suggest the possibility that Zeleny and Senay discarded the large sperms and that the two smaller classes were mixed to some extent producing the result which could not be explained.

II. The Differentiation of the large Generations of Sperm.[8]

As I have stated in the preceding section, the nuclear and cytoplasmic components of the large and small cells behave in a very similar manner throughout the early stages of spermatogenesis and up to the formation of the definitive spermatids. Even during the differentiation of the sperm the cytoplasmic elements show little if any distinctive differences in the large and small cells (see Bowen '22*a*). The spermatid nuclei, on the other hand, in addition to differences in size, exhibit others of a rather unique character. The discovery of these differences early in my study of hemipteran testes, led me to think that the differentiation of the large sperm heads differed fundamentally from that of the smaller ones. However, intensive study of the "normal" spermiogenesis brought to light certain features which had been previously overlooked by workers in this field, and finally provided the data for harmonizing the apparent differences. The formation of the *normal* sperms in *Murgantia* has been described in detail in the second of these "Studies," and it is my purpose here to point out especially those features wherein the development of the *large* sperms is different. The period of spermiogenesis will be divided into a series of *stages* the exact limits of which are given in another place (Bowen '22*a*).

The description is taken from *Murgantia histrionica*, because in this form the relatively small size of the large sperms makes possible the obtaining of complete (or nearly so) heads in sections,— a difficult matter when the heads become very long, as in *Euschistus* for example. I have not examined the situation in other genera very thoroughly, but so far as my observations go, the essential features seem everywhere to accompany the formation of large sperms. In one respect the large spermatids are less satisfactory for study because a complete

[8] In comparing the plates from my study on the normal sperms of *Murgantia* (Bowen '22*a*) with those accompanying this paper, it should be noted that the figure magnifications are somewhat different. In the first named paper the magnification of the plates is 3000, in this paper, 2700.

series of stages cannot be obtained at one time in a single lobe, due primarily to lack of available space. The unusual length of the sperm heads is an added difficulty. The main outlines are, however, quite clear enough for the present purpose, and the account given is drawn largely from a single animal in which the cysts formed a fairly representative series of stages.

Stages l and m. From the formation of the spermatid to the casting off of the acroblast.— These stages do not differ markedly from the corresponding ones in the large cells. The nuclei are larger and appear clearer, the chromatic substance seeming to undergo more complete dissolution than in the normal spermatids. During the early elongation of the halves of the divided nebenkern, the centrioles, appearing in the form of two rods in "V" formation as in the normal spermatids, can be made out (Fig. 11), but clear cases are not frequent because of the relation of the usual plane of section to the long axis of the spermatid nuclei. It is clear, however, that the general history of the centrioles is like that in the normal spermatids. The pseudoblepharoplast is formed in the typical way and the acrosome arises in connection with the Golgi apparatus (acroblast) which is then cast off exactly as in the small sperm (Fig. 12). (Bowen '22*a*).

The aspect of the peripheral chromatin layer, which gradually develops on the inner surface of the nuclear membrane is, however, clearly different, and serves at once to differentiate the large from the small generations. This layer is never thick in the large nuclei, forming a very thin layer (Fig. 12) similar in extent to that in the small nuclei. This thin lining is, furthermore, not at all homogeneous but appears more or less "vacuolated," so that internally the contour is often noticeably rough. In spermatids of the small generation this layer also exhibits some unevenness when the stain is not too heavy, but it is by no means so characteristic as in the case of the large spermatids. The comparison is interesting, however, because it shows that the conditions in both generations are essentially alike.

Stage n. From the migration of the acrosome to its definitive position at the tip of the head, to the inauguration of the final steps in the condensation of the nuclear material.— The first part of stage *n* follows the same general lines as in the small spermatids, the acrosome becoming applied to one surface of the head and developing anteriorly a darkly staining (with hematoxylin) granule; simultaneously the head begins to elongate (Figs. 13 and 14). The most striking divergence from the small sperms is again the appearance of the peripheral chromatic layer, which is now distinctly irregular and often very uneven on its

inner surface. In Figure 15 this irregularity can be clearly seen (in optical section), and this unevenness is reflected in the surface views in which the chromatic layer is very unevenly stained, giving the impression of vacuoles (Fig. 15). Of rather regular occurrence at this time is the appearance at one side of the head and about midway of its long axis of a clump of chromatic material (Fig. 15), which will be again referred to presently. The most striking feature, perhaps, is the absence of any groove formation along the head, so characteristic of the normal sperm heads (see Bowen '22*a*). This groove does not appear ever to be formed at any subsequent stage,— at least in a fairly representative series of cross-sections I have failed to find it at all.

The pseudoblepharoplast now disappears (Fig. 16) as in the normal cases, but from this point on the course of events is otherwise strikingly different. The chromatic layer becomes very highly 'vacuolated' (Fig. 16), and a tendency is noticed toward the formation of what appear to be two large vacuoles, one located in front of, the other behind the mid-axial clump of chromatic material which was forming in Figure 15. The chromatic layer is pretty well broken up, though a definite zone seems to be retained along one side of the head as shown on the anterior vacuole in Figure 16. This stage is a very characteristic one in *Murgantia*, and is particularly striking because of the bizarre appearance given by the chromatic arrangements. Apparently this condition is of very short duration, for the two clear areas soon fade out and the whole head appears stained in an indefinitely irregular way except for a clear area at the base corresponding to the same characteristic of the normal sperm. The head elongates very rapidly especially during the latter part of this stage, and it is during this period that the great difference in length between the large and small sperm heads is established. The arrangement of the chromatic material is simply the same indefinite, vacuolated condition characteristic of the earlier stages (Fig. 17). Whether the chromatin remains peripheral or becomes distributed through the cavity of the head is not known. This peculiar arrangement of the chromatin persists into the next stage and is probably directly comparable to the vacuolation of the chromatin which I have described in the normal sperm heads of *Murgantia* (Bowen '22*a*).

Stage o. The condensation of the chromatic material to form the definitive sperm head.— In stage *o* the chromatic material undergoes a process of condensation the result of which is to produce a sperm head in structure essentially like that of the normal sperms. As in

them (see Bowen '22*a*), the chromatin seems to contract toward the mid-line of the nuclear cavity, and there condenses to form a single, thread-like axis. This is subject to various peculiarities in the early stages, and it is in respect to these that the large sperm heads seem to differ most conspicuously from the smaller ones. In many (all?) cases, this thread is first formed as an irregular, usually incomplete helical thread, or at least such seems to be its shape (Fig. 18, which does not show the tip of the head). Sometimes this seems to be merely irregular chromatin masses, and occasionally cysts occur in which the chromatin is more or less broken up into a series of distinct, well separated, beadlike masses. I do not know what rôle these may play, or, indeed, whether they are to be considered part of the normal procedure. The first mentioned condition is not unlikely an early stage in the formation of the axial thread of chromatin; while the latter condition is perhaps connected with the end stages in the same process.

In any event, this irregular thread soon becomes straightened out, probably in part by its further condensation, and the head of the future sperm is thus produced, corresponding remarkably with the structure of the sperm head of normal size. Through the center of the former nuclear cavity passes a smooth core of chromatin, which is at first enclosed in a layer of protoplasm corresponding to the limits of the old nucleus (Fig. 19).[9] I have never been able to follow this layer over an entire sperm, for obvious reasons, but originally it probably forms a complete mantle as in the small sperm. A characteristic vacuole (perhaps two) is also formed along the head during the final steps, as in the normal sperm, and its origin and fate is doubtless the same, though uncertain, in both cases. During the final condensation of the chromatin thread the anterior part of the head often has a cork-screw shape, as though an originally coiled thread were just in process of straightening out.

Stage p. The sperm completed.— Finally, the outer protoplasmic envelope disappears as in the normal sperm, and the completed sperm (Fig. 20) is now ready to be released into the efferent duct of the testis, a protoplasmic mass having been already sloughed off the tail after the manner customarily followed in normal sperm formation. In stages *o* and *p*, the tips of the heads are so very delicate and so closely bunched (in sections) that it is quite impossible to separate a single one from its neighbors. I have accordingly added the anterior part of the sperm

[9] Compare with Figures 168 and 174 (Bowen '22*a*); the Figures 51, 53, 54 and 55 of *Paludina* given by Meves ('03) are also interesting in this connection.

in Figure 20 more or less from the indications of its length given by the bunch as a whole. Smear preparations show the actual length to be somewhat greater.

The study of smears gives a much better idea of the mature sperm than sections, since the former method furnishes an abundance of complete, isolated heads. Sperm heads from smears (Fig. 21) show one very marked difference from those in sections, the width of the head being apparently much less. The thickness of the head in sections is doubtless largely due to the stain, which not infrequently makes dense chromatic structures appear larger than they really are, especially if the extraction of the stain is not extreme. Two other points of difference are also brought out by smears. One of these is the exceedingly long and delicate (its width is much exaggerated in Fig. 21) whip-like lash at the tip of the head, terminating in a minute, slightly elongate thickening. This lash is presumably of acrosomal origin. The other point is the definite expansion of the basal end of the head (Fig. 21), which seems sometimes to stain more intensely than the rest of the head. This probably corresponds to the somewhat similar portion at the base of the normal sperm heads (see Fig. 177, Bowen '22*a*), for which reason I believe that it probably represents the centrioles ("centrosomal middle-piece"). This would fall in line with the fact already noted, that the centrioles in the large cells are proportionately larger, while the available chromatic material is constant in amount.

The structure of the sperm tail was described in my second "Study," but one feature of the tail of the large sperm calls for additional comment. I refer to the enlargement already noted as a constant occurrence near the tip of the tail (Text-figs. 3*B* and 5*C*). Smears fixed in osmic acid fumes indicate that this enlargement is due to two bleb-like swellings on the threads, probably of mitochondrial (nebenkern) origin, which pass along either edge of the ribbon-like tail (Fig. 22). The meaning and fate of this swelling is unknown. Figure 22 also shows very clearly how the marginal threads gradually narrow into the termination of the sperm tail.

The features described in this section as accompanying the formation of large sperms, are as constant in occurrence as is the polymegalous condition itself. In only one case (a specimen, No. 6, of *Murgantia*), have I found a departure from this rule. In this individual, the early stages are similar to those in the normal sperm, even including the formation of the *groove* along one side of the head. But at some point in the elongation of the head, the sperm revert to the

usual course of events and in the characteristic stages of large sperm formation, the appearances are normal for the large sperm. It is possible that this exception was due to a disturbance in the customary amount of difference between the large and small generations of spermatocytes, of which there were some slight indications.

Conclusion.

In the preceding section the cause and meaning of polymegaly have not been considered, because thus far I have been unable to obtain any evidence bearing on either of these points. Montgomery first ('98) concluded that the larger spermatocytes were "due to their receiving a greater amount of food, . . . That is to say," they "must be nourished by a richer blood supply." Subsequently ('10), he modified the latter statement, stating that the nutritional differences "are due to differences in the follicular nurse cells of the testis." This explanation really explains nothing, for we are still in the dark as to the reasons why certain follicular cells should become larger than their neighbors. Indeed, it is not evident why the enlargement of the nurse cells may not as well be a result as the cause of the large generations of spermatocytes. In any event, the morphological condition of the nurse cells helps us not at all to understand the actuating mechanism behind the whole phenomenon. Furthermore, the unusual case cited in the preceding section of an individual in which cysts of cells normal in size occur together with cysts of large cells in the same lobe, would seem to indicate that nutritional differences are not the only factor at work.

It may be noted here that other cases of polymorphism are known to occur in the male germ cells of several animals, and such differences may be of more general occurrence than we suspect. The most interesting of these cases, from the standpoint of this study, is that described by Blackman ('05) in *Scolopendra*, a myriapod in which two kinds of spermatocytes (and spermatids), differing remarkably in size, occur. Two sizes of sperms are produced, and in general the case reminds one strongly of 'polymegaly' in the pentatomid germ cells. There is, however, one noteworthy difference in the arrangement of the large and small generations, which in *Scolopendra* are not confined to particular follicles or cysts, but occur side by side in all parts of the testis. Blackman was inclined to regard the size differences as due to differences in the supply of nutriment traceable to the spatial relations

of the cells. The formation of two different kinds of spermatozoa (eupyrene and apyrene) in Lepidoptera has long been known, and a somewhat different case (eupyrene and oligopyrene sperms) in certain (prosobranch) molluscs is equally familiar. (See especially Meves '03.) Less well known cases have been reported by Holmgren ('01) and Voinov ('02) in Coleoptera. According to the former, in *Staphylinus* there are two kinds of primary spermatocytes which originally differ greatly in volume, but at the time of maturation are of equal size. The resulting sperms are likewise all of equal size, but according to Holmgren, even though they resemble each other completely, on the basis of their genetic inequality they must also be morphologically and physiologically unequal. This case is of particular interest because, as is evident, the conditions are exactly the reverse of those in the Hemiptera (*Pentatomidae*). According to Voinov, in *Cybister* there are two kinds of spermatogenesis giving rise to sperms of different morphological value. The two processes in this case are distinct, occurring at different seasons of the year. Finally, in *Rana*, two sizes of cells have long been known to occur in the gonads of many of the larvae, the larger cells having been interpreted as abortive eggs. The large spermatocytes of *Arvelius* might, indeed, easily be interpreted in a similar way, were it not for their further history and environment. Swingle ('21) has recently suggested that the two sizes of cells are in reality two generations of spermatocytes, an interpretation which falls in line with the other cases noted above. It is possible that all of these cases are really only scattered instances of a widespread tendency toward polymorphism in sperm cells and are perhaps ultimately to be explained on grounds more or less remotely similar. In any event, the phylogenetic explanation offered by Swingle seems less probable in the light of the other facts which I have here assembled.

There is, in all these cases, the common characteristic that the phenomena peculiar to each seem to be perfectly normal and constant for the particular species involved. Whether the sperms thus formed are all able to function in any of the processes of normal fertilization is not clear. It is indeed certain that they do not in the Anura, and probably also in the case of the apyrene sperm of Lepidoptera in which the nucleus is lacking. Whether the polymegalous sperms of Hemiptera are of equal value in fertilization is not known, and the evidence for their retention in the seminal receptacle of the female after copulation is not conclusive. The matter would be well worth settling, for it would throw a most interesting side-light on the question of the relative value of nuclear and cytoplasmic contributions in fertilization.

The points brought out in this study may be briefly summarized: —

1. Di- or poly-megalous sperms are not confined to the genus *Euschistus*, as Montgomery believed, but are of frequent occurrence throughout the Family *Pentatomidae*.

2. As a result, generations of at least two or three (possibly more) sizes of sperms are developed.

3. The differences in cell-size are due to increase of *all* the constituents of the cell except the *chromatin*, which is *constant* in amount, at least within *very* narrow limits.

4. The large sperms are differentiated by a process superficially unlike, but fundamentally similar to, that which the small sperms undergo.

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Explanation of Plates.

All of the figures have been outlined as far as possible with the camera lucida at an initial enlargement of approximately 3800 diameters. At so great an enlargement it has, of course, been necessary to correct the outlines extensively and to add much of the finer detail free hand. In reproducing, the figures have been reduced uniformly to an enlargement of approximately 2700 diameters. In every case the method employed in the preparation of the original object has been indicated.

A, acrosome
a, acroblast
b, chromatoid body
C, chromatin nucleolus
c, centrioles
G, Golgi remnant
N, nebenkern
P, plasmosome

PLATE I.

Figures 1 and 2 are from *Arvelius albopunctatus;* Figures 3, 4, 7 and 8 are from *Euschistus euschistoides;* Figures 5 and 6 are from *Rhytidolomia senilis;* Figures 9 and 10 are from *Chlorochroa uhleri* (= *persimilis*). The large and small cells in each pair are from the same testis in every case. Figures 2, 4, 6, 8 and 10 are from cells of the large generations; the others are from cells of one of the smaller generations.

1 and 2 Primary spermatocytes in late growth period. (Flemming-hematoxylin.)

3 and 4 Spermatids, stained to show the nebenkern. (Flemming without acetic plus conc. nitric acid-hematoxylin.)

5 and 6 Spermatids, stained to show the chromatoid body. (Flemming-hematoxylin.)

7 and 8 Spermatids, impregnated to show the Golgi apparatus. (Modified Kopsch.)

9 and 10 Spermatids, stained to show the acrosome just after the casting off of the acroblast. (Flemming-hematoxylin.)

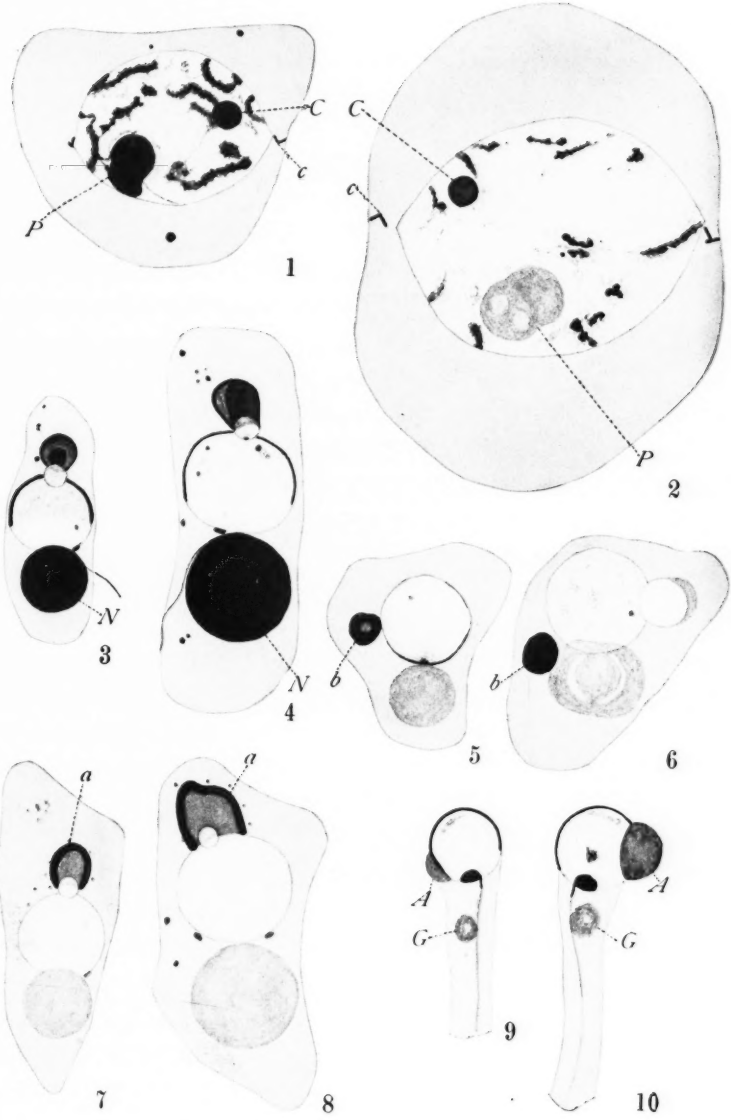
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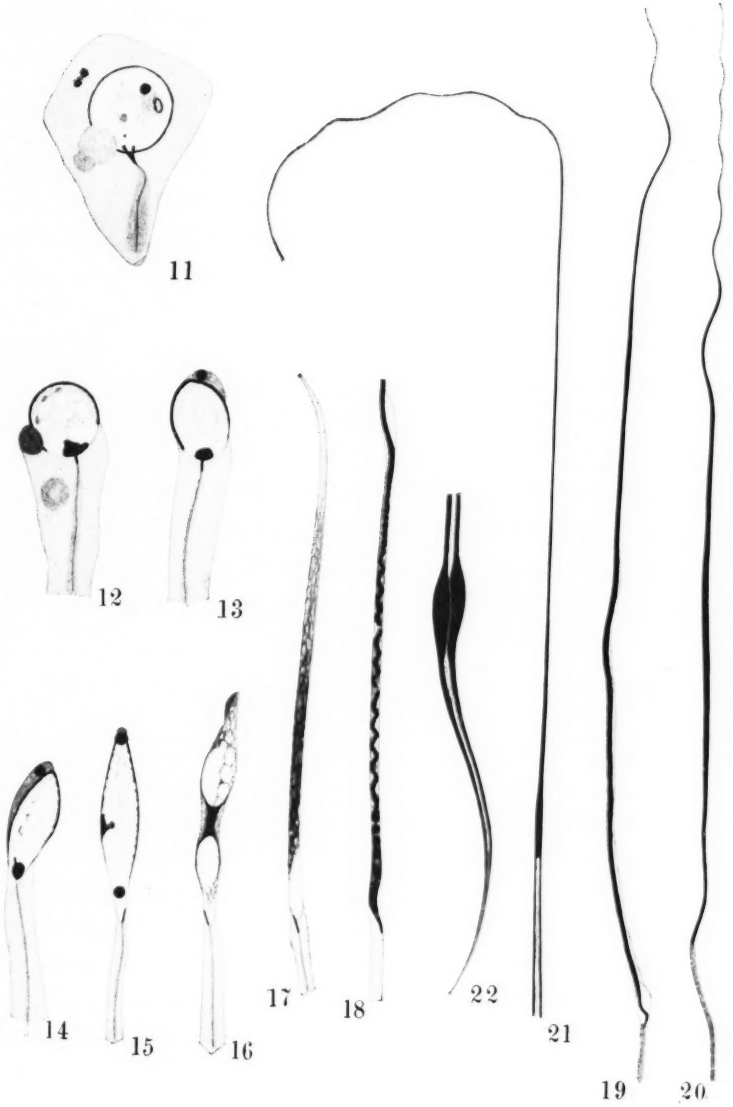
PLATE II.

All the figures are from cells of the large generations in *Murgantia histrionica*.

11 to 20 Progressive stages in the differentiation of the sperm head (nucleus). (Flemming-hematoxylin,— except Figure 20 which is Hermann-hematoxylin.)

21 Head of mature sperm from efferent duct of testis. (Smear preparation. Osmic fumes-hematoxylin.)

22 Detail of the bleb-like swelling on the tail of the large sperms. (Smear preparation. Osmic fumes-hematoxylin.)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